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Adenosine deaminase and xanthine oxidase levels in multiple sclerosis patients

Adenosine Deaminase, Xanthine Oxidase, Multiple Sclerosis patients

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Abstract
Aim: Multiple sclerosis represents an autoimmune, chronic, inflammatory, and demyelinating disease of the central nervous system. Inflammation and oxidative stress are considered to take a significant part in its pathogenesis. Adenosine deaminase (ADA) and xanthine oxidase (XO) enzymes, which are involved in purine metabolism, are critical for regulating inflammation and oxidative stress. Therefore, this study's goal is to evaluate the levels of these two enzymes in patients with the relapsing-remitting type of multiple sclerosis (MS) in their remission period.
Material and Methods: Thirty patients with relapsing-remitting multiple sclerosis (RRMS) who were in their remission period and diagnosed in accordance with the Mc Donald 2010 criteria along with 30 healthy volunteer controls, matched with regard to age and gender, were enrolled in the research. Serum ADA levels were studied by the sensitive colorimetric method that was defined by Giusti, while XO levels were studied by the Worthington method.
Results: RRMS patients had significantly higher serum ADA and XO levels compared to the control subjects (both of the P values = 0.004).
Discussion: In our study, we conclude that two of the most crucial underlying pathogenic mechanisms of MS, inflammation and oxidative stress, may be associated with the increased levels of ADA and XO, and an approach of targeting the activity of these two enzymes can be considered in treatment strategies. Furthermore, we also demonstrated that ADA and XO enzymes were elevated even in the remission phases of RRMS, reflecting the continuity of inflammation through the whole course of RRMS. Thus, in this disease, which is thought to have a dynamic process, the importance of continuous immunomodulatory treatment is emphasized once again.

Keywords
Multiple Sclerosis, Adenosine Deaminase, Xanthine Oxidase

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Introduction

Multiple sclerosis is an autoimmune, chronic, inflammatory, and demyelinating disease of the central nervous system. It constitutes the most frequent cause of disability after trauma in young adults. Multiple sclerosis (MS) represents a heterogeneous neurological disease with multifactorial etiologies characterized by demyelination, axonal degeneration, and oligodendroglial death. There are various forms of MS, e.g. primary progressive (PPMS), secondary progressive (SPMS), and relapsing-remitting multiple sclerosis (RRMS). RRMS represents the most frequently observed form (80%) and is characterized by neurological deficits, generally followed by complete or nearly complete improvements [1]. Although oxidative stress and inflammation are thought to be involved in the etiopathogenesis of the disease, it has not been clearly elucidated yet.

Purinergic signals contribute to the regulation of immune response and inflammation. Extracellular ATP exhibits a proinflammatory effect by stimulating lymphocytes and increasing cytokine release. On the other hand, adenosine as the product of destruction, has a strong anti-inflammatory and immunosuppressive effect by inhibiting T-cell proliferation and cytokine release [2].

Adenosine deaminase is an enzyme that takes an essential part in purine metabolism and is closely related to the regulation of extracellular adenosine levels. Adenosine deaminase (ADA) is a purine metabolism enzyme that catalyzes the irreversible hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively [3].

The formation of hypoxanthine occurs in the subsequent reaction. Xanthine oxidase(XO) catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid. It is a major enzyme that plays a role in the production of reactive oxygen species (ROS) [4].

Xanthine dehydrogenase and XO are Xanthine oxidoreductase(XOR) forms, which can be transformed into each other. While Xanthine dehydrogenase (XDH) plays an active role in the metabolism of hypoxanthine to uric acid under normal conditions, this reaction is carried out by XO because of the transformation of XDH to XO due to hypoxia or ischemia, and molecular oxygen is used as an electron acceptor [5]. The XOR system produces ROS, such as superoxide, nitric oxide, and hydrogen peroxide [6]. ROS are transformed into products, such as peroxynitrite, that are highly toxic to cells, and they cause the main components of the cell, such as lipids, proteins, and nucleic acids, to be damaged, and as a result, lead to cell death by necrosis or apoptosis [7]. ROS, MS, and experimental autoimmune encephalopathy have been found to be responsible for demyelination and axon damage. There is an increasing number of pieces of evidence that both enzymes are involved in the pathogenesis of MS [6]. We could not encounter any study evaluating ADA and XO levels in MS subjects at the same time. The present research aimed to assess purine metabolism, which takes an essential part in inflammation and oxidative stress, through ADA and XO enzyme levels in MS subjects in the remission period (clinically and radiologically inactive period) to avoid the triggering effects of MS attacks on the levels of enzymes which mentioned above.

Material and Methods

Thirty RRMS patients, who were followed up in Cumhuriyet University, Faculty of Medicine, Department of Neurology and who were diagnosed with MS in accordance with the Mc Donald 2010 criteria, were enrolled in the research. They had no history of attacks in the last three months and were receiving interferon treatment, and 30 healthy volunteers, matched with regard to age and gender, were enrolled in the research as a control group. Individuals who were on immunosuppressive drugs and who had diabetes, renal failure, acute infection, and other autoimmune diseases were excluded from the study from both of the groups, the patient and control.

Approval for the study protocol was obtained from the Human Ethics Committee of Cumhuriyet University, Faculty of Medicine (16.02.2016, 2016-02/04). Informed consent was acquired from all patients and healthy volunteers.

Blood collection

Venous blood specimens (10ml) were drawn from RRMS patients and the control group into Vacutainer tubes. The centrifugation of blood specimens was performed at 4000 rpm at a temperature of 4 °C for a period of 15 minutes. Subsequently, the serum specimens obtained were stored at -80 ° C until the ADA and XO levels were studied.

Measurement of enzyme activities

Adenosine deaminase activity was determined by the sensitive colorimetric method defined by Giusti, while XO activity was identified by the Worthington method. The analysis results were presented as specific activity (unit per milligram of protein). Adenosine and hypoxanthine were provided by Sigma Aldrich (Steinheim, Germany).

Statistical Analysis

The data acquired from the research were uploaded into the Statistical Package for Social Sciences (SPSS) (ver. 22.0) program. The Kolmogorov-Smirnov test for normality was used to determine whether the continuous variables had a normal distribution. In the intergroup comparisons of the continuous variables (ADA and XO levels, age), the independent two-samples t-test was used. The corrected Chi-square test was used for the purpose of comparing the groups in terms of gender rates. The variables were expressed as mean ± standard deviation (SD). The value of $p < 0.05$ was accepted as statistically significant.

Results

Thirty RRMS patients in remission and 30 healthy volunteers as a control group were enrolled in the research. Of the RRMS patients, 23 (76.7%) were female and 7 (23.3%) were male. Of the individuals in the control group, 20 (66.7%) were female, and 10 (33.3%) were male. While the average age of the patients was 36.50 ± 7.92, the average age of the control subjects was 35.23 ± 10.65 (Table 1). There was no significant difference with regard to gender and age between the patients and the control subjects ($p>0.05$).

In RRMS patients, the mean Expanded Disability Status Scale (EDSS) score was found to be 1.98 ± 1.49, and no correlation was determined between the severity of MS and ADA and XO values.

The ADA levels were found to be 0.73 ± 0.28 U/mg protein in the RRMS group, which were significantly higher in comparison

with the value of 0.54 ± 0.19 U/mg protein in the control subjects (p = 0.004).

The XO levels were found to be 1.07 ± 0.16 U/mg protein in RRMS patients, which were significantly higher when compared to the value of 0.67 ± 0.11 U/mg protein in the control subjects (p = 0.004) (Table 1).

Table 1. Characteristics and serum ADA and XO levels of the RRMS and control groups

	RRMS group (n)	Control group (n)	P-value
Male	7(23.3%)	10(33.3%)	0.23
Female	23(76.7%)	20(66.7%)	0.42
The mean age	36.50±7.92	35.23±10.65	0.19
EDSS score	1.98 ± 1.49	-	-
ADA(U/mg protein)	0.73±0.28	0.54±0.19	0.004
XO(U/mg protein)	1.07±0.16	0.67±0.11	0.004

Discussion

MS is a disability-causing disease in young adults, by which 2.5 million people are affected worldwide [7]. Therefore, it has a large denominator in the current treatment research. Along with its etiopathogenesis, researchers are continuing to search for molecules that can be affective in MS treatment. In all autoimmune diseases, there is a loss of self-tolerance and control of inflammation [8]. Purine metabolism is one of the most important systems in inflammation regulation. For this reason, there are plenty of studies on the results of purine metabolism enzymes and substrates with diseases such as rheumatoid arthritis, autoimmune hepatitis, and systemic lupus erythematosus. Some similar studies have been and are being carried out in MS [9]. In the purinergic system, ATP is considered as a molecule with pro-inflammatory effects, such as lymphocyte stimulation and proliferation, increasing the release of pro-inflammatory cytokines [10]. On the other hand adenosine is a powerful anti-inflammatory and immunosuppressive metabolite [11]. ADA catalyzes adenosine to inosine by deamination and performs the regulation of extracellular adenosine concentrations [12]. This enzyme has been shown to take an essential part in lymphocyte function and is crucial for the normal growth, proliferation, and differentiation of T-lymphocytes [13].

In one of the studies, Polachini et al. studied products included in purine metabolism, such as ATP, ADP, adenosine, as well as ADA. They examined changes in the enzymes and substrates involved in the inflammatory process and their effects on each other and emphasized that this approach might be the treatment option [14]. In their study, as in our research, the ADA serum levels were found to be significantly higher in patients with RRMS when compared with the control group. As these patients were clinically and radiologically stable when evaluated with the currently available methods, these results show that inflammation continues even in the remission phase of RRMS.

In the study conducted by Samuraki and presenting an MS case, it was reported that the ADA level was elevated in both the cerebrospinal fluid and serum [15]. This is compatible with the breakdown of anti-inflammatory adenosine and the pro-inflammatory process. Contrary to our conclusion, there are also studies in the literature indicating decreased ADA activities in patients with RRMS.

In this situation, it can be worthwhile to increase adenosine with a compensatory mechanism against inflammation and to decrease ROS, such as free oxygen radicals, in the path leading to XO and uric acid [16].

Being effective in steps after the ADA enzyme, XO contributes to the production of ROS, such as superoxide [6]. These substances are also thought to be responsible for the pathogenesis of MS [17].

In the current research, the serum XO levels were also found to be high, which supported the presence of inflammation, such as serum ADA levels. Along with MS, high levels of XO are figured in diseases such as stroke, myelopathy and menengitis when compared with control subjects [18].

In a study in which experimental autoimmune encephalomyelitis was induced in mice, it was found that the XO levels were high on both CSF and serum of these mice [6].

The current treatments are most effective in suppressing inflammation, but their effect in preventing neurodegeneration is insufficient and controversial. Relapses are treated with corticosteroids and immunomodulatory therapies prevent relapses therefore they reduce disability and improve the quality of life of the patients [19].

Since 25-40% of RRMS subjects are transformed into SPMS within 15 years after the onset of the disease, new treatment options that can prevent this conversation are one of the most important unmet needs in this field [20].

Oxidative stress (OS) represents a crucial factor for the pathogenesis of the disease and in all of it's stages. It initiates inflammatory processes in the acute phase and maintains neurodegeneration in the chronic phase [21]. The use of antioxidants in the course of the disease can be effective by reducing the severity of the disease, providing faster remission, and causing less neuroinflammation and neurodegeneration. The transcription factor Nrf2 represents a key regulator of antioxidative defense. Oral dimethyl fumarate (DMF) used in the treatment of MS performs the activation of anti-inflammatory and antioxidative pathways by increasing the expression of Nrf2 [22]. In MS subjects, higher levels of OS markers and lower levels of antioxidant molecules were observed independently of the course of the disease [23]. Febuxostat is a non-purine, selective inhibitor of XO which is currently used for gout treatment . As well as inhibiting the synthesis of uric acid, febuxostat reduces XO-mediated ROS generation and enhances mitochondrial function. In mice, elevated XO levels were found in the experimental autoimmune encephalomyelitis model, and the XO inhibitor, febuxostat, was demonstrated to be effective [6].

As a result, purine metabolism takes an important place in MS pathogenesis with both inflammation and oxidative stress. The increased ADA activity may have led to both decreased anti-inflammatory response and increased inosine level, leading to the possibility of activating the XOR system. Oxidative stress, which takes an essential part in the pathogenesis of MS, may

be more harmfull in neurodegeneration than inflammation. The results of the current study show that neurodegeneration process continues even in the remitting phase of RRMS and the continuous need of immunmodifying treatments through the whole course of RRMS. We conclude that the serum levels of ADA and XO are possible candidates as markers to support MS diagnosis and to show the level of neurodegeneration. Accordingly, testing the effect of new treatment options on the serum levels of ADA, XO and adenosine can be promising.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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References
1. Fox R, Bethoux F, Goldman M, Cohen J A. Multiple sclerosis: advances in understanding diagnosing and treating the underlying disease. Cleve Clin J Med. 2006;73(1):91–102.
2. Burnstock G. An introduction to the roles of purinergic signalling in neurodegeneration, neuroprotection and neuroregeneration. Neuropharmacology. 2016;104:4-17.
3. Cordero OJ, Salgado FJ, Fernández-Alonso CM, Herrera C, Lluis C, R Franco R, et al. Citokines regulate membrane adenosine deaminase on human activated lymphocytes. J Leukoc Biol. 2001;70(6):920-30.
4. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. Int J Cardiol. 2016; 15(213):8-14.
5. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol. 2004;555 (3):589-606.
6. Honorat JA, Kinoshita M, Okuno T, Takata K, Koda T, Tada S, et al. Xanthine oxidase mediates axonal and myelin loss in a murine model of multiple sclerosis. PLoS One. 2013;8(8):e71329.
7. Browne P, Chandraratna D, Angood C, Tremlett H, Baker C, Taylor B, et al. Atlas of Multiple Sclerosis 2013: a growing global problem with widespread inequity. Neurology 2014;83(11):1022-24.
8. Valesini G, Gerardi M, Iannuccelli C, Pacucci V, Pendolino M, Shoenfeld Y. Citrullination and Autoimmunity. Autoimmun Rev. 2015;14(6): 490-7.
9. Sari RA, Taysi S, Yilmaz O, Bakan N. Correlation of Serum Levels of Adenosine Deaminase Activity and Its Isoenzymes With Disease Activity in Rheumatoid Arthritis. Clin Exp Rheumatol. 2003;21(1):87-90.
10. Bours MJ, Swennen EL, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5' triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. Pharmacol Ther. 2006;112(2):358-404.
11. Haskó G, Cronstein B. Regulation of Inflammation by Adenosine. Front Immunol. 2013; 4(85):1-8.
12. Franco R, Casadó V, Ciruela F, Saura C, Mallol J, Canela EI, et al. Cell surface adenosine deaminase: much more than an ectoenzyme. Prog Neurobiol. 1997;52(4):283-94.
13. Khodadadi I, Abdi M, Ahmadi A, Wahedi MS, Menbari S, Lahoorpour F, et al. Analysis of serum adenosine deaminase (ADA) and ADA1 and ADA2 isoenzyme activities in HIV positive and HIV-HBV co-infected patients. Clin Biochem. 2011;44(12):980-3.
14. Polachini CR, Spanevello RM, Schetinger MRC, Morsch VM. Cholinergic and Purinergic Systems: A Key to Multiple Sclerosis? J Neurol Sci. 2018;392:8-21.
15. Samuraki M, Sakai K , Odake Y, Yoshita M , Misaki K, Nakada M, et al. Multiple Sclerosis Showing Elevation of Adenosine Deaminase Levels in the Cerebrospinal Fluid. Mult Scler Relat Disord. 2017;13:44-46.
16. Spanevello RM, Mazzanti CM, Schmatz R, Thomé G, Bagatini M, Correa M, et al. The activity and expression of NTPDase is altered in lymphocytes of multiple sclerosis patients. Clin Chim Acta. 2010;411(3-4):210-4.
17. Gonsette RE. Neurodegeneration in multiple sclerosis:the role of oxidative stress and excitotoxicity. J Neurol Sci. 2008;274(1-2):48-53.
18. Stover JF, Lowitzsch K, Kempski OS. Cerebrospinal Fluid Hypoxanthine, Xanthine and Uric Acid Levels May Reflect Glutamate-Mediated Excitotoxicity in Different Neurological Diseases. Neurosci Lett. 1997; 238 (1-2), 25-8.
19. Lopez-Diego RS, Weiner HL. Novel therapeutic strategies for multiple sclerosis a multifaceted adversary. Nat Rev Drug Discov. 2008;7(11):909-25.
20. Yamout BI, Alroughani R. Multiple Sclerosis. Semin Neurol. 2018;38(2):212-225.
21. Chiurchiù V. Novel targets in multiple sclerosis: to oxidative stress and beyond. Curr Top Med Chem. 2014;14(22):2590-9.
22. Suneetha A, Raja Rajeswari K. Role of dimethyl fumarate in oxidative stress of multiple sclerosis: A review. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1019:15-20.
23. Adamczyk B, Niedziela N, Adamczyk-Sowa M. Novel Approaches of Oxidative Stress Mechanisms in the Multiple Sclerosis Pathophysiology and Therapy. In: Ian S. Zagon and Patricia J. McLaughlin, editors. Multiple Sclerosis: Perspectives in Treatment and Pathogenesis. First pubblications. Brisbane, QLD 4122, Australia: Codon Publications; 2017. p.155-173.

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